## BIOCHEMISTRY

# ELECTROCHEMICAL PROPERTIES OF FREE AND SUBSTRATE ANALOG BOUND FORMS OF WILD TYPE AND MUTANT HUMAN GLUTARYL-COA DEHYDROGENASE

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Glutaryl-CoA Dehydrogenase (GCD) is a mitochondrial matrix enzyme responsible for the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA in the degradation pathways of the amino acids, lysine, tryptophan, and hydroxylysine. Like other members of the acyl-CoA dehydrogenase (ACD) family, many of which are involved in fatty acid oxidation, GCD is a tetramer containing FAD as the electroactive center in the active site of each of its subunits. This FAD extracts 2 electrons from the substrate and passes them to the common ACD electron receptor, electron transfer flavoprotein (ETF). Unique to GCD is the subsequent decarboxylation of the oxidized intermediate to create the product, which diffuses out to join in the rest of the fatty acid oxidation sequence.

To explore the electron transfer step in GCD, substrate analogs 3-thiaglutaryl-CoA and 4-nitrobutyryl-CoA were bound to the enzyme in separate spectroelectrochemical experiments. Midpoint potentials were measured and will be compared to those measured for the free enzyme. Mutant GCDs, specifically R94Q, E370Q, and E370D, were separately evaluated for the midpoint potential values of both their free and their analog bound forms. For free enzymes, it appears that midpoint potentials are shifted negatively when an active site positive charge is removed (R94Q), positively when an active site negative charge is removed (E370Q), and very little when the negative charge is moved slightly (E370D). We also do not see significant shifts in potentials from the free enzyme values when the GCDs are bound to 3-thiaglutaryl-CoA. Binding 4-nitrobutyryl-CoA to the active site offers mixed results.